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Our research interest is focused on the development of new strategies to identify genes from chromosome 17. The isolation of genes transcribed from chromosome 17 will provide candidates for the proposed sporadic breast and ovarian cancer genes. We have recently reported a method for the isolation of chromosome specific cDNAs using high density arrayed cDNA and chromosome specific cosmid libraries. The ability to isolate genes in a chromosome specific manner provides simultaneous identification of the expressed sequence and a chromosomal location. This new technology identifies expressed sequences by reciprocal probing of arrayed cDNA libraries and a chromosome specific cosmid library. We have used these resources to identify 1794 clones from the Los Alamos chromosome 17 cosmid library using probes generated from a placental cDNA library. So far, we have isolated and characterized 42 cDNAs to chromosome 17. Several of these cDNAs mapped to the region 17p13, while 14 other cDNAs mapped to the region of 17q12-22. Recent studies have demonstrated that in some sporadic breast cancer there are loss of heterozygosity (LOH) in these regions of human chromosome 17.

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Introduction

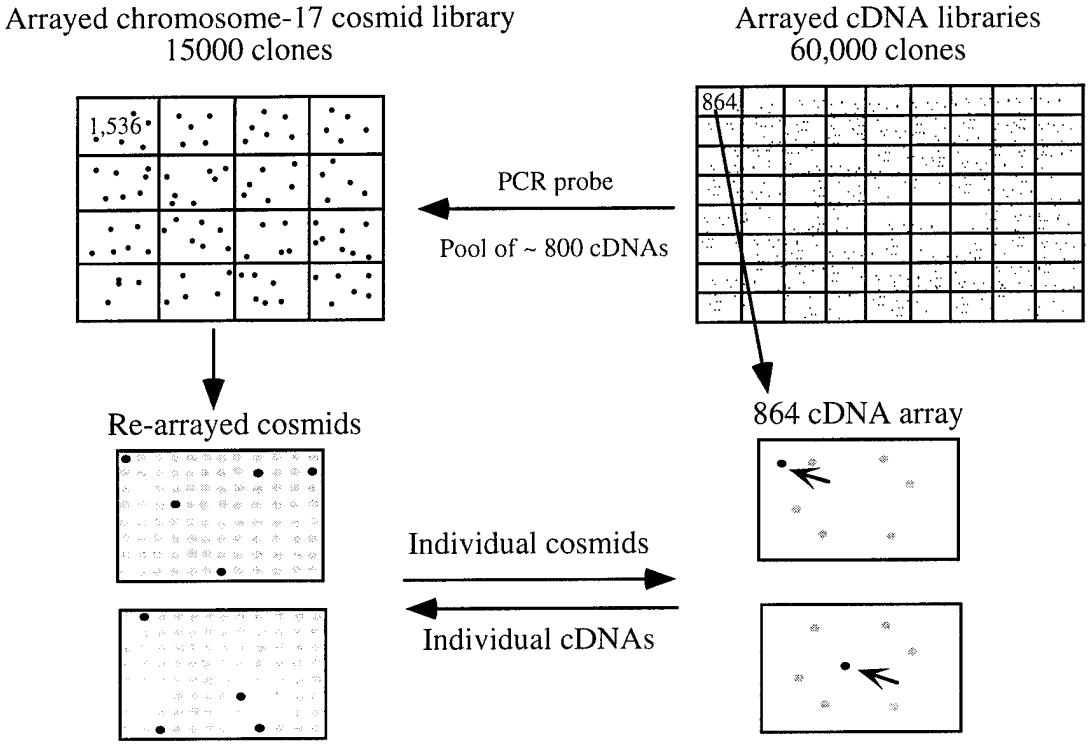
The role of genetics in cancer is now well established with the identification of several genes where the presence of mutation has been associated with cancer formation (1). One of the most exciting of these includes a gene for hereditary breast cancer, namely BRCA1. This gene has also been associated with hereditary cancer of the ovary (2,3). The gene for BRCA 1 was mapped to chromosome 17q21, a region that is also associated with allele losses (loss of heterozygosity, (LOH)) in sporadic breast and ovarian cancer (4,5,6,7,8,9). After intensive investigation by many research groups, the gene for BRCA 1 was recently identified (10). Mutation in BRCA 1 was found in many patients with hereditary breast and ovarian cancer (11,12). Surprisingly, these studies also show that mutations in BRCA1 are rare in sporadic breast and ovarian cancers that are thought to be due to susceptibility to the disease at this locus (13,14,15). Together, the LOH studies and the lack of mutation in BRCA 1 gene have led to the proposal that there is another gene within this region of 17q12-q22 that is associated with sporadic breast and ovarian cancer in women (16). Recent studies have demonstrated that LOH in other regions of human chromosome 17 is also associated with breast cancer (17,18). These studies have indicated that a region telomeric to the P53 gene at 17p13.3 (about 3 cM) is also believed to harbor a separate tumor suppressor gene associated with breast cancer. Given the enormous effort expanded to find BRCA 1, the challenge to identify these two potential sporadic breast cancer genes on the chromosome 17 is expected to be great. Until recently, the strategies for cloning a disease-related gene included either functional or positional cloning approaches (19). The gene for BRCA 1 was identified by positional cloning methods (10). With the effort of the Human Genome Initiative in cDNA and expressed sequence tag (EST) mapping, a candidate gene approach to finding human disease genes has been predicted to be the future trend. Our research interest is focused on the development of new strategies to identify genes from chromosome 17. The isolation of genes transcribed from chromosome 17 will provide candidates for the proposed sporadic breast and ovarian cancer genes.

Experimental methods and results

We have recently reported a method for the isolation of chromosome specific cDNAs using high density arrayed cDNA and chromosome specific cosmid libraries (20). The ability to isolate genes in a chromosome specific manner provides simultaneous identification of the expressed sequence and a chromosomal location. This new technology identifies expressed sequences by reciprocal probing of arrayed cDNA libraries and a chromosome specific cosmid library. The strategy for reciprocal probing of complex arrayed libraries is shown in Figure 1.

Figure. 1

Reciprocal probing of arrayed cDNA and cosmid libraries



Strategy for reciprocal probing of arrayed cDNA and chromosome-specific cosmid libraries. Cot-1 DNA hybridization signals are symbolized by the dots on the arrayed cDNA library filters. cDNA pools were radiolabeled and hybridized to the arrayed cosmid filters. Between 80 and 150 specific cosmid clones were identified from both LL0XNC01 "U" and LA17NC01 libraries after each hybridization. These cosmids were rearrayed in a 96-well format and copies of these arrayed clones were generated on filters for parallel rescue of expressed sequences. Individual cosmid DNA was used as a probe on the arrayed cDNA filter that corresponded to the cDNA pool used to identify this cosmid. The arrows on the cDNA array point to the dots that symbolize the specific cDNA hybridizations, while the background signals are generated by cDNAs with repeat elements. The cosmid-specific cDNA was back-hybridized to the re-arrayed cosmid filter to identify the corresponding and overlapping cosmids as symbolized by the black dots.

Human tissue cDNA libraries were constructed in lambda ZAP II vector (21) using *Not* I linkers. Clones from these primary cDNA libraries were deposited into 96-well trays for storage and retrieval. Vector sequence hybridization between cDNA and cosmid libraries was minimized by generating polymerase chain reaction (PCR)-based cDNA elements from the arrayed clones. A primer pair (each 25 bases) flanking the *Not* I cloning site of the lambda ZAP II vector was used to amplify cDNA elements in a 96-well reaction format using a "hot start" PCR approach with an aliquot of the arrayed lambda ZAP cDNA clones as template. The amplified cDNA elements were arrayed for hybridization onto nylon filters using a Beckman Biomek-1000 robot. The PCR-based cDNA products provided two advantages: a rapid method for generating cDNA inserts to use as probes, and the creation of vectorless target DNA from the arrayed cDNA library for hybridization.

Repeat elements like Alu and Line sequences are present on average every 5 kb of human genomic DNA (22,23). Hybridization of our arrayed cDNA libraries to Cot-1 DNA (24) showed that 5–10% of the cDNAs contain repeat elements. In order to increase the specificity and sensitivity of cDNA probes to be pooled, hybridization signals generated by repeat elements had to be overcome. Conventionally, repeat sequence hybridization is reduced by preassociation of the probe with high levels of human or Cot-1 DNA. Because of the relatively high frequency of repeat elements in genomic and expressed sequences, blocking by human DNA alone was not sufficient. In contrast, by virtue of cDNA library being arrayed, clones containing repeat elements were identified by Cot-1 DNA hybridization. Aliquot of PCR-based cDNAs without repeat elements were then pooled and radiolabeled as probes for screening chromosome-specific cosmid libraries. Common and unique cosmid hybridization signals were observed, indicating that a complex pool of cDNA probes could be used to scan cosmids containing genomic sequences for expressed sequence homologies. The identification of specific cosmid-cDNA associations by hybridization was carried out in one step. Our prior identification of individual cDNAs with repeat sequences by Cot-1 DNA hybridization and the knowledge of their grid positions on the arrayed filters minimized the study of cosmid-cDNA associations due to repeat sequence hybridization. By comparing specific cosmid to Cot-1 DNA probe hybridization patterns, unique sequence associations were readily identified (Fig. 1). Hybridization signals of repeat elements were therefore used as interpretative tools to identify specific cDNA-cosmid associations.

By reciprocal probing of arrayed cDNA filters with the cosmids, cDNAs associated with respective cosmids were identified. These cDNA clones were sequenced through one pass sequencing from the 5' and 3'ends. The corresponding cosmids were used for fluorescent in situ hybridization (FISH) mapping to localize their chromosomal position. The sequence information was used to generate STS primers for polymerase chain reaction (PCR) mapping on chromosome 17 somatic hybrid cell-lines to further confirm the cDNA and the corresponding cosmid map position.

To date we have focused on the placental cDNA library. We have generated cDNA elements by PCR from 20,000 clones of the placenta library. These cDNA elements were then pooled and used as probes on the high density chromosome 17 cosmid library. We have identified 1794 cosmids that hybridized to these placental probes. We are in the process of isolating the associated cDNA to these cosmids as described in the methods. Based on the assumption that the cosmid library has at least 5X coverage of the human chromosome 17, the cosmids we have identified may be associated with up 300 genes. So far, from the placental cDNA library, 42 unique cDNAs of chromosome 17 have been identified and mapped (see Table 1). Additionally, another 69 cDNA-cosmid associations have been established but we have limited information on these genes and they will not be discussed in this report. To date we have kept up with the 5' and

Table 1: Novel and described chromosome 17 genes. The cDNA accession numbers were provided by the Los Alamos Genome Sequence Database (GSDB. The column " Description" shows tha database entry with the highest significant homology and the corresponding accession number. The mapping category 1 indicates consistency between FISH and STS mapping, while category 2 indicates either STS mapping is positive either on mapping panel or associated cosmid. While category 3 indicates only hybridization based mapping was achieved. n/a, not available. N.D, not determined; P; published chromosome 17 genes

Sr. No.	Accession No.	cDNA	Cosmid	GSDB Sequence Homology		Map position	Mapping Category
				Description	Acc. No.		
1	L32006/07	6B4	54D8	Pigment epithelium- derived factor	M76979	17p13	2a,P
2	L31972/73	15D8	46D4	C. elegan C50C3	L14433	17p13	1
3	L31986/87	24F3	66F9	Rat Proteasome	D10754	17p13	1
4	L31974/77	15H4	86D8	EST00870	M78722	17p13	1
5	L31998/99	2G6	131E6	Human eIF-4A1	D13748	17p12	1
6	L32011/12	22E12	137C9	Novel	None	17p12	1
7	L32004/05	5G12	59E8	Human eEF-1A	J04617	17p11.2	2a
8	L31968/69	13G2	90E12	Novel	None	17q11.2-q12	1
9	L32009/10	13C11	11D3	Novel	None	17q11.2-q12	1
10	L32013/14	25B11	65H12	Human Cytokeratin-19	J03607	17q12	1,P
11	L31975/76	18G2/9G12	93C11	Human Cytokeratin-8	X74929	17q12-21.1	1
12	L31982/83	22A1	17B7	Novel	None	17q12-21.1	1
13	L31984/85	22D3	23G6	EST06635	T08743	17q12-q21.1	1
14	L31990/91	2A11/2E8	3A12	Human HMG-17 Protein	X13546	17q21.1	2a
15	L31994/95	2D12	4C6	Novel	None	17q21.1	1
16	L31988/89	26C7	156A2	Novel	None	17q21.3	1
17	L32002/03	5F2	113G1	Human Granulin	M75161	17q21.3	1,P
18	L31980/81	21H5	27B11	Novel	None	17q22	1
19	L32008/15	12G12	18E12	Novel	None	17q22	1
20	L31996/97	2F12	9C4	EC-8	M25773	17q23	1
21	L32040/41	2D9	50B10	Human Gamma-Actin	X04098	17q23.1-qter	2b,P
22	L31978/79	20B2	61B7	Rat Histone H3	X73683	17q24.3-q25.1	1
23	L32000/01	4F4	44H2	Novel	None	17q24.3-q25.1	1
24	L31992/93	2A2	112D3	Human Mac-2 Binding Protein	L13210	17q25	1
25	L32067/68	12D8	79C9	Novel	None	17q25	3
26	L31970/71	14B6	54H5	Novel	None	17q25	1
27	None	8B11	122A10	Human β4-Integrin	X52186	17q25.1-q25.2	P
28	None	4B12	121D4	Human Chorionic Somatomammotropin	M15894	N.D.	P
29	L32057/537	6G6	86D12	Human Cytokeratin-18	X12883	N.D.	P
30	None	11H6	17A12	Human B12 mRNA	M80783	N.D.	P
31	None	15C8	139G11	Human L19 Ribosome protein	S56985	N.D.	P
32	L39924	14E8	84D6	Novel	None	17q21.3-q22	3
33	n/a	43H9	29H9	Novel	None	17p12	1
34	n/a	44B3	127C12	L35a Ribosomal protein		17q11.2	
35	n/a	43B6	141B12	EST		17q25	
36	n/a	45B5	21F1	Novel		17p13	
37	n/a	41G7	145G12	ARS		17p11.2	1
38	n/a	65A10	5C2	Flil		17p11.2	P
39	n/a	65F4	138D7	Novel		17p11.2-p12	
40	n/a	76G7	47G7	Novel		17p11.2	
41	n/a	50D9	12H11	Protein tyrosine phosphatase		17q21	P
42	n/a	63B4	20G2	Heme A:farnesyltransferase		17q12	

3' sequencing of the cDNAs isolated. The information obtained from the 5' and 3' ends of these cDNAs has allowed for the search against the Genome data base for sequence homology. Of the characterized cDNAs, 20 of these are either novel or have homology to ESTs in the data base; 11 cDNAs have previously been described and mapped to chromosome 17; the remaining cDNAs are homologous to previously described but unmapped genes. Four cDNAs mapped to the region 17p13, while another 14 cDNAs are mapped to the region of 17q12-22, both of these regions are targeted for LOH in sporadic breast and ovarian cancers. Therefore, these cDNAs are possible candidate genes for the proposed sporadic breast cancer genes.

Conclusions

The proposal goals for the past year focused on the development of resources and refinement of technology so that genes specific to chromosome 17 could be identified rapidly. To date we have successfully generated the resources proposed for this project. These include the completion of the 40,000 clones arrayed placental and ovarian cDNA libraries. The generation of PCR products for high density filters from the placental library is complete while that of the ovarian library is in progress. This phase of the project was achieved primarily by manual effort. At no cost to this grant, we have acquired a Beckman-1000 robot for generating high density filters needed for this proposal. The Beckman-1000 robot will automate the task involved in generating high density filters and expedite the progress of this project. We have used these resources to identify 1794 clones from the Los Alamos chromosome 17 cosmid library using probes generated from the placental cDNA library. The number of chromosome 17 genes isolated is projected to increase significantly with the completion of the arraying activity. So far, we have isolated and characterized 42 cDNAs to chromosome 17. Several of these cDNAs mapped to the region 17p13, while 14 other cDNAs mapped to the region of 17q12-22. So far, none of the 14 cDNAs located within 17q12-22 encode for BRCA 1. However, two of these cDNAs, 22A1 and 50D9 were identical to recently described genes located very near BRCA 1 (25, 26). The cDNA 50D9 encode for a protein tyrosine phosphatase whose homolog in rat is PRL-1 (27). The PRL-1 protein tyrosine phophatase have been shown to alter cellular growth and morphology. The region between 17q21-22 is very gene rich and the use of the placental cDNA library may have bias against the identification of BRCA 1. However, given the fact that BRCA 1 has been identified, we will now focused on other genes for possible involvement in sporadic breast cancer. Given the fact the LOH on chromosome 17 is also present in sporadic ovarian tumor, the genes isolated from the ovarian cDNA will be deeply characterized. We proposed to determine message RNA size and expression by northern blotting for genes mapped to the LOH regions. We also proposed to isolate the full-length cDNA to these genes. Recently, my laboratory has obtained direct access to an ABI

373A automated fluorescence sequencer. This has allowed increased flexibility and capacity in DNA sequencing. To enhance the isolation of chromosome 17 genes being expressed from breast tissue, we proposed for an arrayed breast cDNA library. To limit disruption to current research, the breast cDNA library will not be used until the completion of our current goals.

The present study demonstrated that this novel strategy for isolating chromosome specific gene is efficient. The reagents generated by the reciprocal probing strategy including cDNAs, maps cosmids, and sequence tag site (STS) primers can provide a high level of transcript map characterization. The isolation and mapping of chromosome 17 cDNAs has provided candidates for the proposed sporadic breast cancer genes and other human diseases mapped to this chromosome.

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